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APPLICATION NUMBER: *60/458,845*
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By Authority of the
COMMISSIONER OF PATENTS AND TRADEMARKS

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Certifying Officer

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PTO/SB/16 (10-01)
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PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53 (c).

Express Mail Label No. EL474274351US

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Photon	Rao	Millbrae, CA

☐ *Additional inventors are being named on the separately numbered sheets attached hereto*

TITLE OF THE INVENTION (500 characters max)

COMPOSITIONS AND METHODS FOR TREATING CANCER

CORRESPONDENCE ADDRESS

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ENCLOSED APPLICATION PARTS ***(check all that apply)***

☒ Specification *Number of Pages* 33 ☐ CD(s), Number

☐ Drawing(s) *Number of Sheets* ☒ Other (specify) Fee Transmittal

☒ Application Data Sheet. See 37 CFR 1.76

METHOD OF PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT

☒ Applicant claims small entity status. See 37 CFR 1.27.

☐ A check or money order is enclosed to cover the filing fees

☒ The Commissioner is hereby authorized to charge filing fees or credit any overpayment to Deposit Account Number: 20-1430

FILING FEE AMOUNT ($) 80

☐ Payment by credit card. Form PTO-2038 is attached.

The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

☒ No.

☐ Yes, the name of the U.S. Government agency and the Government contract number are:

Respectfully submitted,
SIGNATURE [signature]

Date 03/28/03

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Docket Number: 021305-000900US

USE ONLY FOR FILING A PROVISIONAL APPLICATION FOR PATENT

PA 3295014 v1

60458845 .032803

PTO/SB/17 (01-03)

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FEE TRANSMITTAL for FY 2003

Patent fees are subject to annual revision.

☒ Applicant claims small entity status. See 37 CFR 1.27

TOTAL AMOUNT OF PAYMENT | ($) 80

Complete if Known

Application Number	To Be Assigned
Filing Date	March 28, 2003
First Named Inventor	Matteucci, Mark
Examiner Name	To Be Assigned
Group Art Unit	To Be Assigned
Attorney Docket No.	021305-000900US

METHOD OF PAYMENT (check all that apply)

☐ Check ☐ Credit Card ☐ MoneyOrder ☐ Other ☐ None

☒ Deposit Account:

Deposit Account Number: 20-1430

Deposit Account Name: Townsend and Townsend and Crew LLP

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FEE CALCULATION

1. BASIC FILING FEE

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description	Fee Paid
1001	750	2001	375	Utility filing fee	
1002	330	2002	165	Design filing fee	
1003	520	2003	260	Plant filing fee	
1004	750	2004	375	Reissue filing fee	
1005	160	2005	80	Provisional filing fee	80

SUBTOTAL (1) ($)80

2. EXTRA CLAIM FEES FOR UTILITY AND REISSUE

			Extra Claims		Fees from below		Fee Paid
Total Claims		-** =		X		=	
Independent Claims		-** =		X		=	
Multiple Dependent				X		=	

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description
1202	18	2202	9	Claims in excess of 20
1201	84	2201	42	Independent claims in excess of 3
1203	280	2203	140	Multiple dependent claim, if not paid
1204	84	2204	42	** Reissue independent claims over original patent
1205	18	2205	9	** Reissue claims in excess of 20 and over original patent

SUBTOTAL (2) ($)

**or number previously paid, if greater; For Reissues, see above

FEE CALCULATION (continued)

3. ADDITIONAL FEES

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description	Fee Paid
1051	130	2051	65	Surcharge - late filing fee or oath	
1052	50	2052	25	Surcharge - late provisional filing fee or cover sheet.	
1053	130	1053	130	Non-English specification	
1812	2,520	1812	2,520	For filing a request for reexamination	
1804	920*	1804	920*	Requesting publication of SIR prior to Examiner action	
1805	1,840*	1805	1,840*	Requesting publication of SIR after Examiner action	
1251	110	2251	55	Extension for reply within first month	
1252	410	2252	205	Extension for reply within second month	
1253	930	2253	465	Extension for reply within third month	
1254	1,450	2254	725	Extension for reply within fourth month	
1255	1,970	2255	985	Extension for reply within fifth month	
1401	320	2401	160	Notice of Appeal	
1402	320	2402	160	Filing a brief in support of an appeal	
1403	280	2403	140	Request for oral hearing	
1451	1,510	1451	1,510	Petition to institute a public use proceeding	
1452	110	2452	55	Petition to revive – unavoidable	
1453	1,300	2453	650	Petition to revive – unintentional	
1501	1,300	2501	650	Utility issue fee (or reissue)	
1502	470	2502	235	Design issue fee	
1503	630	2503	315	Plant issue fee	
1460	130	1460	130	Petitions to the Commissioner	
1807	50	1807	50	Petitions related to provisional applications	
1806	180	1806	180	Submission of Information Disclosure Stmt	
8021	40	8021	40	Recording each patent assignment per property (times number of properties)	
1809	750	2809	375	Filing a submission after final rejection (37 CFR § 1.129(a))	
1810	750	2810	375	For each additional invention to be examined (37 CFR § 1.129(b))	
1801	750	2801	375	Request for Continued Examination (RCE)	
1802	900	1802	900	Request for expedited examination of a design application	

Other fee (specify) ____________________

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Express Mail No.: EL474274351US

PA 3295009 v1

Application Data Sheet

Application Information

Application number::	To Be Assigned
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Petition included?::	No
Secrecy Order in Parent Appl.::	No

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60458845 .032803

Attorney Docket No.: 021305-000900US
Express Mail No.: EL474274351US

PROVISIONAL

PATENT APPLICATION

Compositions and Methods for Treating Cancer

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As Filed March, 2003

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Compositions and Methods for Treating Cancer

FIELD OF THE INVENTION

[0001] The present invention provides methods, compounds, and compositions useful in the treatment of cancer and so relates to the fields of chemistry, medicinal chemistry, pharmacology, and medicine.

BACKGROUND OF THE INVENTION

[0002] The term "cancer" generally refers to one of a group of more than 100 diseases caused by the uncontrolled growth and spread of abnormal cells that can take the form of solid tumors, lymphomas, and non-solid cancers such as leukemia. Unlike normal cells, which reproduce until maturation is attained and then only as necessary for replacement, cancer cells grow and divide endlessly, crowding out nearby cells and eventually spreading to other parts of the body, unless their progression is stopped. Once cancer cells metastasize by leaving a tumor, they will travel through the bloodstream or lymphatic system to other parts of the body, where the cells begin multiplying and developing into new tumors. This sort of tumor progression makes cancer dangerously fatal. Although there have been great improvements in diagnosis, general patient care, surgical techniques, and local and systemic adjuvant therapies, most deaths from cancer are still due to metastases and other cancers that are resistant to conventional therapies including radiation and chemotherapy.

[0003] Radiation therapy is typically only effective for cancer treatment at early and middle stages of cancer, when cancer is localized, and not effective for late stage disease with metastasis. Chemotherapy can be effective at all stages of the disease, but there can be severe side effects, e.g. vomiting, low white blood cells, loss of hair, loss of weight and other toxic effects, to radiation therapy and chemotherapy. Because of such severe side effects, many cancer patients do not or cannot successfully complete a chemotherapy treatment regimen. The side effects of radiation and anticancer drugs can be viewed as resulting from poor target specificity. Anticancer drugs, typically administered intravenously or more rarely orally, circulate through most normal tissues of patients as well as the target tumors. If the drug is toxic to a normal cell, then this circulation will result in the death of normal cells, leading to side effects, and the more toxic the drug to normal cells, the more serious the side effects.

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Express Mail No.: EL474274351US

Due to these and other problems, some highly cytotoxic chemotherapeutic agents, agents with nanomolar or sub-nanomolar IC_{50} values against cancer cells, have not been successfully developed into approved drugs.

[0004] Prodrugs have been investigated as a means to lower the unwanted toxicity or some other negative attribute of a drug without loss of efficacy. A prodrug is a drug that has been chemically modified to render it inactive but that, subsequent to administration, is metabolized or otherwise converted to the active form of the drug in the body. For example, in an effort to improve drug targeting, prodrugs have been developed that are activated under hypoxic conditions. Hypoxia creates a bioreductive environment, and certain anti-cancer agents have been converted into prodrugs that can be activated in such environments. See the reviews by Naylor *et al.*, May 2001, *Mini. Rev. Med. 1*(1):17-29, and Denny, 2001, *Eur. J. Med Chem. 36*: 577-595. "Hypoxia" is a condition of low oxygen levels; most solid tumors larger than about 1 mm in diameter contain hypoxic regions (see the references Coleman, 1988, *J. Nat. Canc. Inst. 80*: 310; and Vaupel *et al.*, *Cancer Res. 49*: 6449).

[0005] As a tumor grows, it requires a blood supply and thus the growth of new vasculature. The new vasculature that supports tumor growth is often highly unordered, leaving significant portions of the tumor under-vascularized and subject to intermittent vascular blockage. The vascular architecture of the tumor can contribute significantly to the cancer's ability to survive drug therapy in at least two different ways. First, if the drug must reach the cancer through the bloodstream, then not as much drug will reach the under-vascularized, hypoxic areas of the tumor. Second, to the extent the drug requires oxygen to be effective, then the drug will be less effective in the hypoxic regions of the tumor.

[0006] Conversely, however, the hypoxic environment is conducive to reductive events that can be used to generate reduced derivatives of a variety of chemical groups (see the reference Workman *et al.*, 1993, *Cancer and Metast. Rev. 12*: 73-82), and bioreductive prodrug compounds have been developed to exploit such environments. These prodrugs include the antibiotics Mitomycin C (MMC) and Porfiromycin (POR), N-oxides such as Tirapazamine (TRZ; see the reference Zeeman *et al.*, 1986, *Inst. J. Radiot. Oncol. Biol. Phys. 12*: 1239), quinones such as the indoloquinone E09 (see the reference Bailey *et ål.*, 1992, *Int. J. Radiot. Oncol. Biol. Phys. 22*: 649), cyclopropamitosenes (EP-A-0868137), and a tertiary amine-N-oxide analogue of Mitoxantrone (AQ4N) that is activated by cytochrome P450 3A4

(see the references Patterson, 1993, *Cancer Metast. Rev. 12*: 119; and Patterson, 1994, *Biochem. Pharm. Oncol. Res. 6*: 533).

[0007] Other bioreductively activated prodrug compounds include the nitroimidazole derivatives that have been reported to be useful in cancer radiotherapy as radio-sensitizing agents (see the patent publications EP312858 and WO91/11440) and potentiators of chemotherapeutic agents (see U.S. Patent No. 4,921,963). Nitroimidazole has also been conjugated to the anti-cancer agent PARP 5-bromoisoquinolinone (see the reference Parveen et al., Jul. 1999, *Bioorg. Med. Chem. Lett., 9*:2031-36). The nitroimidazole moiety itself is, however, somewhat cytotoxic to normal cells, because it undergoes redox cycling and generates superoxides under oxygenated conditions.

[0008] Thus, there remains a need to provide drugs to treat cancer. Such drugs would be especially beneficial if they targeted cancer cells more effectively than current drugs and had fewer, less serious side effects. The present invention helps meet this need.

SUMMARY OF THE INVENTION

[0009] The present invention provides compounds, compositions, and methods for treating cancer.

[0010] In one aspect, the present invention provides methods for treating cancer in a subject, comprising administering to the subject an effective amount of a hypoxia-activated prodrug of the invention, alone or in combination with another anti-cancer agent or therapeutic treatment, including surgery and radiation.

[0011] In another aspect, the present invention provides compounds that are hypoxia-activated prodrugs having a structure defined by the formula: Hyp-L-N, wherein Hyp is a hypoxic activator; L is a bond or a linking group or linker; and N is an anti-neoplastic agent.

[0012] In one embodiment, the hypoxic activator is selected from the group consisting of an electron deficient nitrobenzene, an electron deficient nitrobenzoic acid amide, a nitroazole, a nitroimidazole, a nitrothiophene, a nitrothiazole, a nitrooxazole, and a nitrofuran. The prodrug can comprise one, two, or more such hypoxic activator moieties. In one embodiment, the bond or linker is an ester, ether, acetal or carbamate.

[0013] In one embodiment, the anti-neoplastic agent N is selected from the group consisting of cytotoxic agents having an IC_{50} less than 100 microM, and optionally less than 1 microM, as defined by the NCI screening assay as an LC_{50} after a 24 hr drug treatment of a sensitive cell line. In some embodiments, the cytotoxic agent has an IC_{50} less than 10 nanomolar. In one embodiment, N is doxorubicin or, when cleaved from the prodrug conjugate in the body, generates a doxorubicin derivative having an IC_{50} in the low nanomolar range. In one embodiment, N is selected from the group consisting of maytansines, enediyenes, discodermolides, epothilones, taxanes, calicheamicins, and tedanolides. In another embodiment, N is selected from the group consisting of etoposide, vinblastine, vincristine, topotecan, cyclphosphamide, 5-fluorouracil, AQ4N, and hydroxyurea.

[0014] In a third aspect, the present invention provides methods for synthesizing the prodrugs of the invention and compounds useful as intermediates in such synthetic methods.

[0015] In a fourth aspect, the present invention provides pharmaceutical formulations of the prodrugs of the invention.

[0016] These and other aspects and embodiments of the invention are described in more detail in the detailed description and claims that follow.

DETAILED DESCRIPTION OF THE INVENTION

[0017] To facilitate an understanding of the invention, the following definitions are provided, and unless defined otherwise, all technical and scientific terms used herein have the meanings ascribed to them by those of skill in the fields to which this invention belongs.

[0018] As used herein, "a" or "an" means "at least one" or "one or more."

[0019] As used herein, an “anti-neoplastic agent”, “anti-tumor agent”, or “anti-cancer agent”, refers to any agent used in the treatment of cancer. Such agents can be used alone or in combination with other compounds and can alleviate, reduce, ameliorate, prevent, or place or maintain in a state of remission of clinical symptoms or diagnostic markers associated with neoplasm, tumor or cancer. Anti-neoplastic agents include, but are not limited to, anti-angiogenic agents, alkylating agents, antimetabolite, certain natural products, platinum coordination complexes, anthracenediones, substituted ureas, methylhydrazine derivatives, adrenocortical suppressants, certain hormones and antagonists, anti-cancer polysaccharides and certain herb or other plant extracts.

[0020] As used herein, an "anti-neoplastic treatment", "cancer therapy", or "cancer treatment" refers to any treatment designed to treat a neoplasm, tumor, or cancer by reducing the number of or growth of cancer cells in the body, typically by killing or halting the growth and division of cancer cells.

[0021] As used herein, a "bioreductive compound" refers to a compound that donates electrons in an oxidation-reduction reaction.

[0022] As used herein, "cancer" refers to a disease caused by a malignant tumor.

[0023] As used herein, "malignant" refers to cells that have the capacity of metastasis, with loss of both growth and positional control.

[0024] As used herein, "neoplasm" (neoplasia) or "tumor" refers to abnormal new cell or tissue growth, which may be benign or malignant.

[0025] As used herein, a "prodrug" is a compound that, after administration, is metabolized or otherwise converted to an active or more active form with respect to at least one property. To produce a prodrug, a pharmaceutically active compound can be modified chemically to render it less active or inactive, but the chemical modification is such that an active form of the compound is generated by metabolic or other biological processes. A prodrug may have, relative to the drug, altered metabolic stability or transport characteristics, fewer side effects or lower toxicity, or improved flavor, for example (see the reference Nogrady, 1985, *Medicinal Chemistry A Biochemical Approach,* Oxford University Press, New York, pages 388-392). Prodrugs can also be prepared using compounds that are not drugs.

[0026] With these definitions, one can better appreciate the various aspects and embodiments of the invention described herein.

[0027] In one aspect, the present invention provides methods for treating cancer in a subject, such as a human or other mammal, said method comprising administering to the subject a therapeutically effective amount of a hypoxia-activated prodrug of the invention, alone or in combination with one or more additional anti-cancer agents or anti-cancer treatments, including but not limited to surgery and radiation.

[0028] In another aspect, the present invention provides hypoxia-activated prodrugs having the formula: Hyp-L-N, wherein Hyp is a hypoxic activator; L is a bond or a linker; and N is an anti-neoplastic agent. Those of skill in the art will appreciate that the anti-

neoplastic agent that is linked to the hypoxic moiety to form the prodrug can be different from the drug that is released from the prodrug upon activation. This aspect of the invention is illustrated in the Examples below, one of which shows that a prodrug of the invention can be synthesized by linking doxorubicin to a hypoxia-activated moiety of the invention to form a prodrug that releases an iminium-containing doxorubicin derivative that is far more toxic than doxorubicin. Thus, while the anti-neoplastic agent N in the formula above can sometimes be the same as the agent used in the synthesis of the prodrug, that term more importantly refers to the anti-neoplastic agent that is released from the prodrug.

[0029] The prodrugs of the invention, relative to the drugs to which they are converted *in vivo*, are much less (at least ten and up to one million-fold less) toxic. The reduced toxicity results from a modification at the site of attachment of the linker L (as in the case where activation of the prodrug releases the same cytotoxic agent that was used in the synthesis of the drug) or from the generation of a moiety required for toxicity by removal of the hypoxic moiety Hyp. In either event, the prodrugs of the invention are converted into the corresponding toxic drug in hypoxic tissues by virtue of the activation of the hypoxic activator moiety, resulting in its removal and the concomitant release or generation of the anti-neoplastic agent.

[0030] In one embodiment, the hypoxic activator moiety Hyp is selected from the group consisting of an electron deficient nitrobenzene, an electron deficient nitrobenzoic acid amide, a nitroazole, a nitroimidazole, a nitrothiophene, a nitrothiazole, a nitrooxazole, and a nitrofuran. The prodrug can comprise one, two, or more such hypoxic activator moieties, which may be the same or different. Preferred hypoxic activators of the invention include nitroimidazoles. Prodrugs containing nitroimidazoles can be used to "mask" (or upon activation, release) an aldehyde, amino, or hydroxyl group of a cytotoxic agent, groups often required for potent toxicity of a cytotoxic agent.

[0031] Nitroimidazoles have been used to form prodrugs of only two putative anti-cancer agents, a PARP inhibitor (see the reference Parveen *et al.*, 1999, *Bioorganic and Medicinal Letters 9*: 2031-2036) and a nitrogen mustard, which was activated, not released, by the nitroimidazole (see the reference Lee *et al.*, 1998, *Bioorganic and Medicinal Letters 8*: 1741-1744). The PARP inhibitor was shown to be released chemically, but no cell culture data was provided. The nitrogen mustard was shown to be active in cell culture data, and the

selectivity between normoxic and hypoxic toxicity, while not accurately measured, was stated as greater than 7 fold in cells with normal DNA repair mechanisms.

[0032] The prodrugs of the present invention differ from such known prodrugs either in the nature of the anti-neoplastic agent released, the better side effect profile, the presence of more than one hypoxia-activated moiety, or some combination of these attributes. These advantages of the prodrug compounds of the present invention can be better appreciated with an understanding of the pharmacokinetics of hypoxia-activated prodrugs generally and those of the invention particularly.

[0033] Nitroimidazole is, in the absence of oxygen, converted to a free radical containing moiety by a cytochrome P450 reductase. If the nitroimidazole is appropriately covalently bound to another moiety, further reduction of the free radical form of nitroimidazole can lead to release of that moiety. However, in the presence of oxygen, the free radical reacts with oxygen to form superoxide and the parent nitroimidazole. Superoxide is a cytotoxin, so the production of superoxide in normoxic tissues is believed to lead to unwanted side effects. The prodrugs of the invention that release "super toxins" can be used in much lower doses than than the nitroimidazole prodrugs heretofore known. These lower doses lead, in turn, to lower production of superoxide in normoxic tissue.

[0034] Certain nitroimidazole-containing prodrugs can also be activated regardless of the oxygen tension by DT diaphorase, which can lead to activation in normoxic cells, thus contributing to unwanted side effects. Should this normoxic activation pathway create significant side effects with a particular prodrug of the invention, however, one can select another prodrug of the invention that contains more than one hypoxia-activated moiety to reduce or eliminate such side effects.

[0035] The prodrugs provided by the present invention are compounds that exhibit greater efficacy and fewer side effects than prior art compounds. For example, certain preferred prodrugs of the invention are conjugated to, or are activated by hypoxic conditions to release, very powerful cytotoxic agents, "super toxins" with IC_{50} values of less than 100 nM against a majority of the cancer cell lines in the NCI tumor cell line panel. Even though the compounds of the invention still generate the superoxide that causes unwanted side effects, those side effects are greatly reduced, because, on a molar basis, much less compound has to be given due to the highly cytotoxic nature of the anti-cancer agent released by the prodrug.

[0036] The prodrugs of the invention can, however, encompass a wide variety of anti-neoplastic agents. In one embodiment, the anti-neoplastic agent (N in the formula above) is selected from the group consisting of cytotoxic agents having an IC_{50} less than 1 microM, optionally less than 100 nanoM. In some embodiments, the IC_{50} of the cytotoxin released from the prodrug of the invention is less than 100 picomolar. In one embodiment, N is doxorubicin or, when cleaved from the prodrug conjugate in the body, generates a doxorubicin derivative having an IC_{50} in the low picomolar range, such as an iminium form of doxorubicin. In one embodiment, N is selected from the group consisting of maytansines, enediyenes, discodermolides, epothilones, taxanes, calicheamicins, and tedanolides. In another embodiment, N is selected from the group consisting of etoposide, vinblastine, vincristine, camptothecin, topotecan, cyclphosphamide, 5-fluorouracil, AQ4N, and hydroxyurea.

[0037] Other illustrative anti-neoplastic agents that can incorporated in or released from the prodrugs of the invention include but are not limited to bleomycins, calicheamicins, colchicine, cyclophosphamide, cytarabine, dacarbazine, dactinomycin, daunorubicin, discodermolides, doxorubicin and doxorubicin-like compounds such as epirubicin and derivatives, enediyenes, epothilones, etoposide, fludarabine, 5-fluorouracil or prodrugs thereof such as Xeloda marketed by Roche, hydroxyurea, hydroxyureapentostatin, maytansines, 6-mercaptopurine, methotrexate, mitomycin, mitoxantrone, platinum-containing agents including but not limited to carboplatin and cisplatin, prednisone, procarbazine, taxanes including but not limited docetaxel and paclitaxel, tedanolides, teniposide, 6-thioguanine, topotecan, and vinca alkaloids including but not limited to vinblastine and vincristine.

[0038] Regardless of the anti-neoplastic agent selected for incorporation in or release by the prodrug, the linker and hypoxic activator moiety are attached to the agent in a manner that effectively masks or reduces its cytotoxic activity. This masking effect can vary and will of course depend on the cytotoxic activity of the anti-neoplastic agent to be released but will minimally be 10 fold or more, including up to one million fold or more, but typically 100 to 10,000-fold. As one example, for an anti-neoplastic agent with an IC_{50} of 1 nM, the corresponding IC_{50} of the prodrug can be 1 microM or greater. Thus, the prodrugs of the invention include prodrugs of any chemotherapeutic agent that can be linked to a hypoxia-activated moiety in a manner that yields a prodrug that is at least 10-fold to 1,000,000-fold,

typically 100 to 10,000-fold, less active as a cytotoxic agent than the drug that is released from the prodrug under hypoxic conditions.

[0039] In addition to contributing to the masking effect and to reducing the cytotoxic activity of the prodrug, the linker or some portion thereof must be capable of being released upon hypoxic activation in a manner that generates the cytotoxic agent from the prodrug. For the preferred hypoxic moieties of the invention, the preferred linker or bond is a carbamate, ether, or acetal. Preferred prodrugs include but not limited to prodrugs having the structure nitroimidazole-$(CH_2)_n$-X, where n is 1 to 5, preferably 1, and X is OCO-anti-neoplastic agent, OCONH-anti-neoplastic agent, OCHOMe-anti-neoplastic agent, or O-anti-neoplastic agent (the prodrugs of the invention can also include the linkers described in the reference Naylor *et al.*, 1992, *J. Med. Chem.* *35*:3573-78).

[0040] Administration of the hypoxia-activated prodrugs of the invention for the treatment of cancer can be effected by any method that enables delivery of the prodrugs to the site of action, the hypoxic region of a tumor. Many cancer drugs are administered by intravenous injection, and the present invention provides formulations of the compounds of the invention suitable for such administration, including not only ready-for-injection formulations but also lyophilized or concentrated formulations that must be rehydrated or diluted, respectively, prior to injection. In addition to these formulations, the present invention provides formulations suitable for administration by oral routes, intraduodenal routes, parenteral injection (including intravenous, subcutaneous, intramuscular, intravascular or infusion), topical, and rectal routes. Those of skill in the art will recognize that the prodrugs of the invention can be activated by bacteria in the gut. If such activation is not desired, then the practitioner may employ a route of administration or a formulation that results in absorption of the prodrug prior to its entry into the large intestine or colon. The actual route of administration and corresponding formulation of the invention will depend on the type of cancer being treated, the prodrug selected for administration, the severity of the cancer, and the age, weight, and condition of the patient, among other factors.

[0041] In similar fashion, the amount of the prodrug administered, and thus the amount of the prodrug contained in the dose administered and the product comprising that dose, will be dependent on the subject being treated, the severity of the cancer, localization of the cancer, the rate of administration, the disposition of the prodrug (e.g., solubility and cytotoxicity), the

cytotoxic agent released by the prodrug, and the discretion of the prescribing physician. However, an effective dosage is typically in the range of about 0.001 to about 100 mg per kg body weight, preferably about 1 to about 35 mg/kg/day, in single or divided doses. For a 70 kg human, this would amount to about 0.05 to about 7 g/day, preferably about 0.2 to about 2.5 g/day. In some instances, dosage levels below the lower limit of the aforesaid range may be more than adequate, while in other cases still larger doses may be employed without causing any harmful side effect; larger doses can also be divided into several small doses for administration throughout the day.

[0042] The anti-neoplastic prodrug composition may, for example, be in a form suitable for oral administration as a tablet capsule, pill powder, sustained release formulation, solution, and suspension; for parenteral injection as a sterile solution, suspension or emulsion; for topical administration as an ointment or cream; and for rectal administration as a suppository. The anti-neoplastic prodrug composition may be in unit dosage forms suitable for single administration of precise dosages and will typically include a conventional pharmaceutical carrier or excipient.

[0043] Suitable pharmaceutical carriers include inert diluents or fillers, water and various organic solvents. The pharmaceutical compositions may, if desired, contain additional ingredients such as flavorings, binders, excipients, and the like. Thus for oral administration, tablets containing various excipients, such as citric acid may be employed together with various disintegrants, such as starch, alginic acid, and certain complex silicates, and with binding agents such as sucrose, gelatin and acacia. Additionally, lubricating agents such as magnesium stearate, sodium lauryl sulfate, and talc can be used to prepare the tablet forms of formulations of the prodrugs of the present invention. Solid compositions of a similar type can be employed in soft and hard filled gelatin capsules. Preferred materials, therefore, include lactose or milk sugar and high molecular weight polyethylene glycols. When aqueous suspensions or elixirs are desired for oral administration, the prodrug therein may be combined with various sweetening or flavoring agents, coloring matters or dyes and, if desired, emulsifying agents or suspending agents, together with diluents such as water, ethanol, propylene glycol, glycerin, or combinations thereof.

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[0044] Exemplary parenteral administration forms include solutions or suspensions of the hypoxia-activated prodrug in sterile aqueous solutions, for example, aqueous propylene glycol or dextrose solutions. Such dosage forms can be suitably buffered, if desired.

[0045] Methods of preparing various pharmaceutical compositions with a specific amount of active prodrug are known, or will be apparent, to those skilled in this art in view of this disclosure. For examples, see Remington's Pharmaceutical Sciences, Mack Publishing Company, Easter, Pa., 15^{th} Edition (1975).

[0046] The prodrugs and pharmaceutical compositions thereof of the present invention can be used in accordance with the methods of the invention to treat any type of cancer in a human or other mammal. Such cancers include but are not limited to leukemia, breast cancer, skin cancer, bone cancer, liver cancer, brain cancer, cancer of the larynx, gallbladder, pancreas, rectum, parathyroid, thyroid, adrenal, neural tissue, head and neck, stomach, bronchi, kidneys, basal cell carcinoma, squamous cell carcinoma of both ulcerating and papillary type, metastatic skin carcinoma, osteosarcoma, Ewing's sarcoma, veticulum cell sarcoma, myeloma, giant cell tumor, small-cell lung tumor, gallstones, islet cell tumor, primary brain tumor, acute and chronic lymphocytic and granulocytic tumors, hairy-cell tumor, adenoma, hyperplasia, medullary carcinoma, pheochromocytoma, mucosal neuronms, intestinal ganglloneuromas, hyperplastic corneal nerve tumor, marfanoid habitus tumor, Wilm's tumor, seminoma, leiomyomater tumor, cervical dysplasia and in situ carcinoma, neuroblastoma, retinoblastoma, soft tissue sarcoma, malignant carcinoid, topical skin lesion, mycosis fungoide, rhabdomyosarcoma, Kaposi's sarcoma, osteogenic and other sarcoma, malignant hypercalcemia, renal cell tumor, polycythermia vera, adenocarcinoma, glioblastoma multiforma, leukemias, lymphomas, malignant melanomas, and epidermoid carcinomas.

[0047] The prodrugs of the invention are of course especially effective against cancers containing significant areas of hypoxic tissue. Such cancers include but are not limited to lung cancer, especially non-small cell lung cancer, breast cancer, colon cancer, head and neck cancer, ovarian cancer, pancreatic cancer, and prostate cancer. Several of these cancers are discussed for illustrative purposes below. Those of skill in the art will appreciate that cancer chemotherapy often involves the simultaneous or successive administration of a variety of anti-cancer agents, and as discussed further below, the prodrugs of the invention can be used

in combination therapies as provided by the methods of the present invention. Thus, in the description of illustrative cancers containing hypoxic regions amenable to treatment with the prodrugs of the invention, illustrative combination therapies provided by the present invention are also described.

[0048] Lung cancer affects more than 100,000 males and 50,000 females in the United States, most of whom die within 1 year of diagnosis, making it the leading cause of cancer death. Current protocols for the treatment of lung cancer involve the integration of chemotherapy with or without radiotherapy or surgery. The hypoxia-activated prodrugs of the invention, including those that release chemotherapeutic agents presently used to treat various forms of lung cancer, can be used to treat lung cancer, for example, by replacing a non-hypoxia-activated form in the combination, and other prodrugs of the invention can be used in existing combination therapies. A variety of combination chemotherapy regimens have been reported for small cell lung cancer, including the combinations consisting of cyclophosphamide, doxorubicin and vincristine (CAV); etoposide and cisplatin (VP-16); and cyclophosphamide, doxorubicin and VP-16 (CAVP-16). Modest survival benefits from combination chemotherapy (etoposide plus cisplatin) treatment have been reported for non-small cell lung cancer. Prodrug forms of each of the chemotherapeutic agents mentioned above are provided by the present invention.

[0049] Likewise, several different cytotoxic drugs have produced at least temporary regression of ovarian cancer. The most active drugs in the treatment of ovarian have been alkylating agents, including cyclophosphamide, ifosfamide, melphalan, chlorambucil, thiotepa, cisplatin, and carboplatin. Current combination therapies for ovarian cancer includes cisplatin or carboplatin in combination with cyclophosphamide at 3- to 4-week intervals for six to eight cycles. The present invention provides prodrug forms of each of these agents, and methods for treating ovarian cancer in which a prodrug of the invention is used in such combinations, either to replace an agent or in addition to the agent(s) currently used.

[0050] Cancer of the prostate is the most common malignancy in men in the United States and is the second most common cause of cancer death in men above age 55, and this cancer has been reported to consist primarily of hypoxic tissue. Several chemotherapy protocols have been reported for use in late stage disease following relapse after hormonal treatment. Agents for the treatment of prostate cancer include estramustine phosphate, prednimustine,

and cisplatin, and prodrug forms of each of these agents is provided by the present invention, as well as methods for treating prostate cancer using such agents. Combination chemotherapy is also used to treat prostate cancer, including treatment with estramustine phosphate plus prednimustine and cisplatin, and 5-fluorouracil, melphalan, and hydroxyurea. The present invention provides prodrug forms of each of these agents, and methods for treating prostate cancer in which a prodrug of the invention is used in such combinations, either to replace an agent or in addition to the agent(s) currently used.

[0051] Cancer of the large bowel is the second most common cause of cancer death in the United States and is likewise a cancer characterized by hypoxic regions. While chemotherapy in patients with advanced colorectal cancer has proven to be of only marginal benefit, 5-fluorouracil is the most effective treatment for this disease. 5-Fluorouracil is useful alone or in combination with other drugs, but is associated with only a 15 to 20 percent likelihood of reducing measurable tumor masses by 50 percent or more. Thus, the hypoxia-activated prodrug form of 5-FU provided by the present invention, and the methods for treating colon cancer using that prodrug, offer significant therapeutic benefit and potential for meeting the unmet need for better treatment methods for this disease.

[0052] As the foregoing discussion illustrates, the present invention provides methods for treating cancer in a human or other mammalian subject, which methods comprise administering to the subject an effective amount of a hypoxia-activated prodrug of the invention in combination with an effective amount of a chemotherapeutic agent (or agents), an effective amount of radiotherapy, an appropriate surgery procedure, or a combination of such therapies. Anti-neoplastic agents useful in combination with one or more prodrugs of the invention for the treatment of cancer include but are not limited to busulfan, improsulfan, piposulfan, benzodepa, carboquone, 2-deoxy-D-glucose, lonidamine, meturedepa, uredepa, altretamine, imatinib, triethylenemelamine, triethylenephosphoramide, triethylenethiophosphoramide, trimethylolomelamine, chlorambucil, chlornaphazine, estramustine, ifosfamide, mechlorethamine, mechlorethamine oxide hydrochloride, melphalan, novembichin, phenesterine, prednimustine, trofosfamide, uracil mustard, carmustine, chlorozotocin, fotemustine, nimustine, ranimustine, dacarbazine, mannomustine, mitobronitol, mitolactol, pipobroman, aclacinomycins, actinomycin F(1), anthramycin, azaserine, bleomycin, cactinomycin, carubicin, carzinophilin, chromomycin, dactinomycin, daunorubicin, daunomycin, 6-diazo-5-oxo-1-norleucine, mycophenolic acid, nogalamycin,

olivomycin, peplomycin, plicamycin, porfiromycin, puromycin, streptonigrin, streptozocin, tubercidin, ubenimex, zinostatin, zorubicin, denopterin, pteropterin, trimetrexate, fludarabine, 6-mercaptopurine, thiamiprine, thioguanine, ancitabine, azacitidine, 6-azauridine, carmofur, cytarabine, dideoxyuridine, doxifluridine, enocitabine, floxuridine, 5-fluorouracil, tegafur, L-asparaginase, pulmozyme, aceglatone, aldophosphamide glycoside, aminolevulinic acid, amsacrine, bestrabucil, bisantrene, carboplatin, defofamide, demecolcine, diaziquone, elfornithine, elliptinium acetate, etoglucid, flutamide, gallium nitrate, hydroxyurea, interferon-alpha, interferon-beta, interferon-gamma, interleukin-2, lentinan, mitoguazone, mitoxantrone, mopidamol, nitracrine, pentostatin, phenamet, pirarubicin, podophyllinic acid, 2-ethylhydrazide, procarbazine, razoxane, sizofiran, spirogermanium, paclitaxel, tamoxifen, teniposide, tenuazonic acid, triaziquone, 2,2',2"-trichlorotriethylamine, urethan, vinblastine, and vincristine.

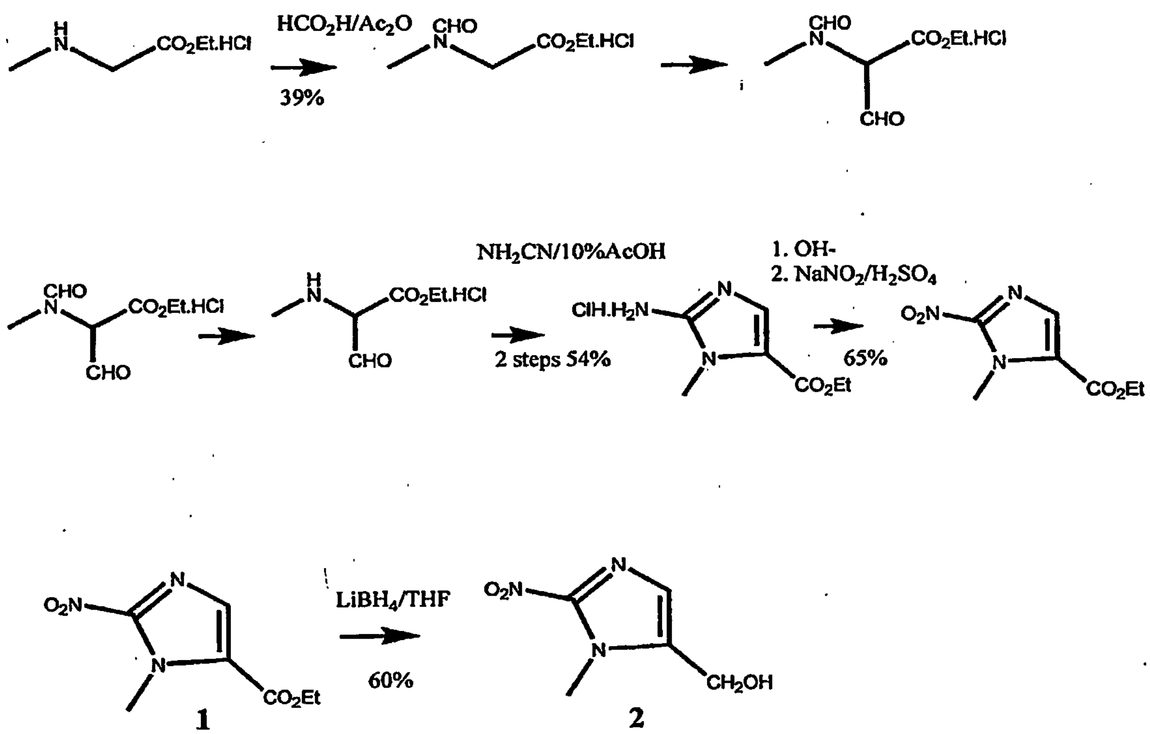
[0053] These and other aspects and embodiments of the invention will be apparent to those of skill in the art upon contemplation of the foregoing disclosure and the illustrative embodiments provided in the following examples and so within the scope of the following claims.

Example 1

Synthesis of Nitroimidazole-based Hypoxia Activator Moieties

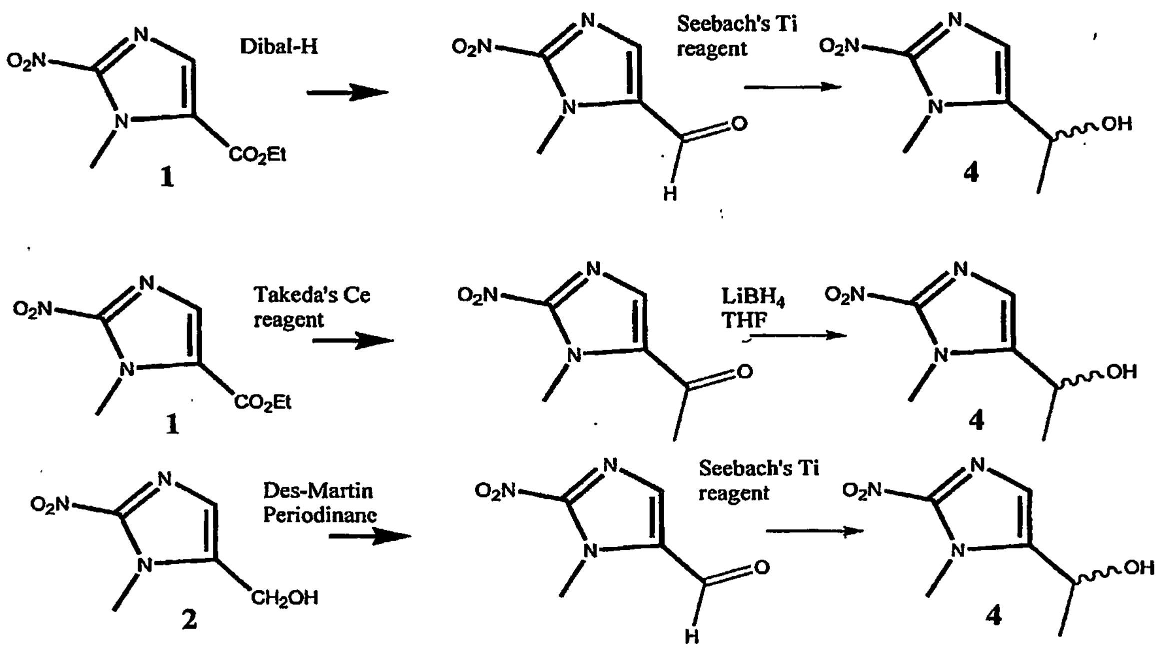
[0054] This example illustrates methods for the synthesis of nitroimidazole-based, hypoxic activator moiety intermediates useful in methods of the invention for synthesizing prodrugs of the invention. In part A, an illustrative method for the synthesis of N(3)-methyl-2-nitro-4-methanol, a compound called herein "the nitroimidazole primary alcohol" from ethyl sarcosine hydrochloride is provided. Because prodrugs of the invention containing this hypoxic activator moiety may in some cells be activated even under normoxic conditions due to the attack on the primary carbon of the alcohol by glutathione-S-transferase or via a similar mechanism, and because further substitution of this carbon can reduce or eliminate such unwanted activation, the present invention also provides the novel intermediate compounds of the invention called herein "the nitroimidazole secondary alcohol" and "the nitroimidazole tertiary alcohol" as well as the novel methods of the invention for their synthesis in parts B and C, respectively.

[0055] Part A. *Synthesis of the Nitroimidazole Primary Alcohol.* The following scheme provides a method for the synthesis of the nitroimidazole primary alcohol (compound 2 in the scheme) from ethyl sarcosine. In this scheme, ethylsarcosine hydrochloride is first converted to ethyl-N-formyl-C-formyl sarcosine hydrochloride; a suitable method for such conversion is described in the reference Jones, 1949, *J. Am. Chem. Soc. 71*: 644, incorporated herein by reference. The latter compound is then converted to a compound called herein "the nitroimidazole ester" (compound 1 in the scheme); a suitable method for such conversion is described in the reference Asato *et al.*, 1972, *J. Med. Chem. 15*: 1086, incorporated herein by reference. Then, the nitroimidazole ester is converted to the nitroimidazole primary alcohol; a suitable method for such conversion is described in Parveen *et al.*, 1999, *Bioorg. Med. Chem. Lett. 9*: 2031, incorporated herein by reference.

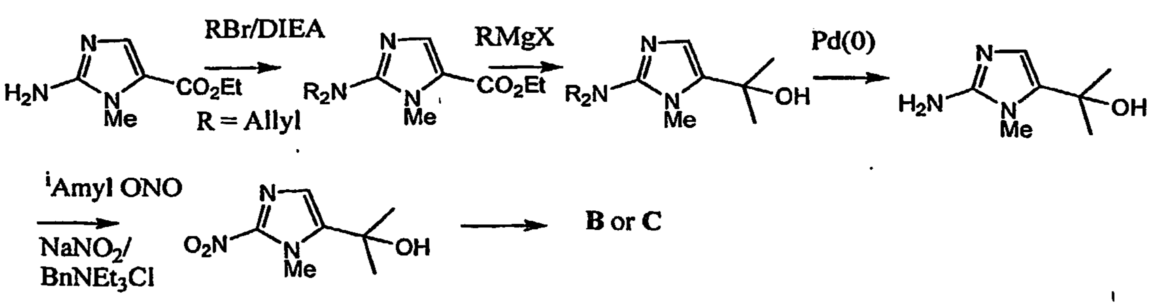


[0056] Part B. *Synthesis of the Nitroimidazole Secondary Alcohol.* As noted above, the present invention provides a novel compound, the nitroimidazole secondary alcohol, and methods for its synthesis. In three different illustrative methods, as shown in the schemes below, either the nitroimidazole ester (compound 1 in the scheme in part A and in the schemes below) or the nitroimidazole primary alcohol (compound 2 in in the scheme in part A and in the schemes below) is converted to the nitroimidazole secondary alcohol using

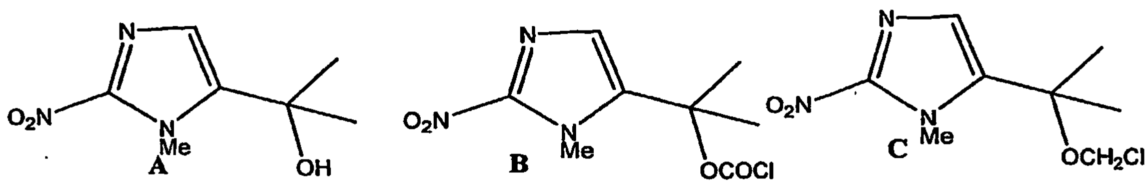
either Cerium reagents (second scheme below; see Takeda *et al.*, *Organic Syntheses*, Volume *76*, page 228 *et seq.* and the references cited therein, incorporated herein by reference) or Titanium reagents (first and third schemes below (see Imwinkelried *et al. Organic Syntheses*, Volume *CV 8*, page 495 *et seq.*, and the references cited therein, incorporated herein by reference).



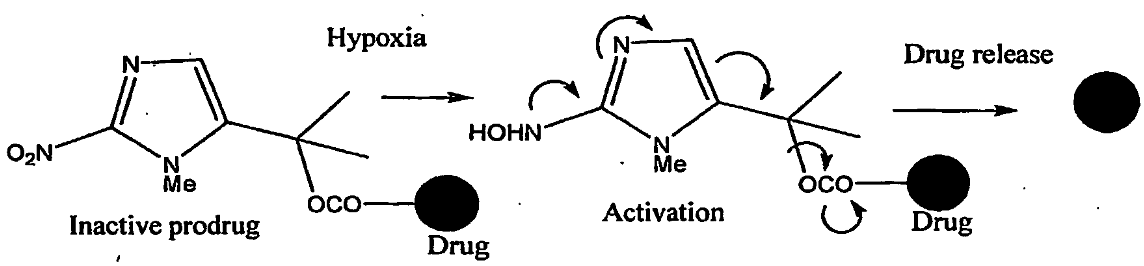
[0057] Part C. *Synthesis of the Nitroimidazole Tertiary Alcohol.* As noted above, the present invention provides a novel compound, the nitroimidazole tertiary alcohol, and methods for its synthesis. In the illustrative method shown in the scheme below, a compound called the aminoimidazole ester (the precursor to compound 1 in the scheme in part A) is converted to the nitroimidazole tertiary alcohol of the invention.



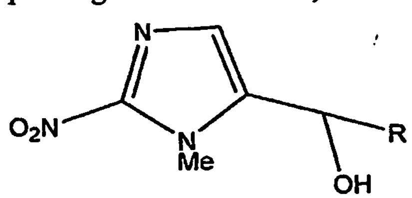
[0058] The nitroimidazole tertiary alcohol (compound A, below) can in turn be converted to the chloroformate (compound B, below), and the chloromethyl ether (compound C, below) compounds of the invention, shown below.



[0059] Compounds A, B, and C, above, are useful as intermediates in the synthesis of the hypoxia-activated prodrugs of the invention, where they serve to block hydroxy, amino, and carboxyl groups in the anti-neoplastic agent to be released from the prodrug. Such prodrugs are activated under hypoxic conditions, because the nitro group in the moiety gets reduced to a hydroxylamine or an amine and releases the drug. This activation process is shown in the following scheme. While the scheme shown illustrates an ester linkage, and such linkages can be present in the prodrugs of the invention, ester linkages are not preferred (preferred linkages include carbamate, ether, and acetal linkages).



[0060] Compounds A, B, and C, above, like the compounds described in parts A and B of this Example (shown as Compounds D and E, below) are thus, when incorporated into a prodrug of the invention, activated by hypoxia.



D R = H
E R = Me

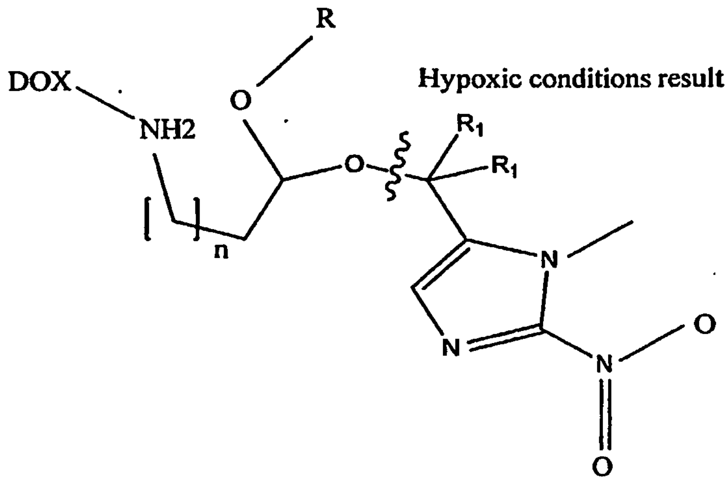
[0061] However, Compounds A, B, and C, above, should be less susceptible to glutathione/glutathione transferase mediated attack, due to the presence of a tertiary, as opposed to the primary (Compound D) or secondary (Compound E), alcohol in those compounds.

Example 2

Prodrugs of Doxorubicin and Related Compounds

[0062] This Example describes illustrative prodrugs of the invention that release highly cytotoxic derivatives of doxorubicin. Those of skill in the art will appreciate that doxorubicin, epirubicin, and daunomycin, and the numerous analogs and derivatives of those compounds that have been and continue to be synthesized represent a class of compounds that can be readily converted into prodrugs of the invention based on the teachings herein. This example illustrates such prodrugs of the invention that release highly cytotoxic compounds under hypoxic conditions. Highly cytotoxic daunomycin derivatives are described in the reference Bakina and Farquhar, 1999, Anti-cancer Drug Design 14: 507, incorporated herein by reference. Part A of this Example illustrates a prodrug of the invention that releases such a highly cytotoxic derivative, referred to herein as a doxorubicin derivative, and that comprises one hypoxia-activated moiety. Part B of this Example illustrates such a prodrug that comprises more than one such moiety. Part C of this Example illustrates such a prodrug that releases a novel dicationic derivative.

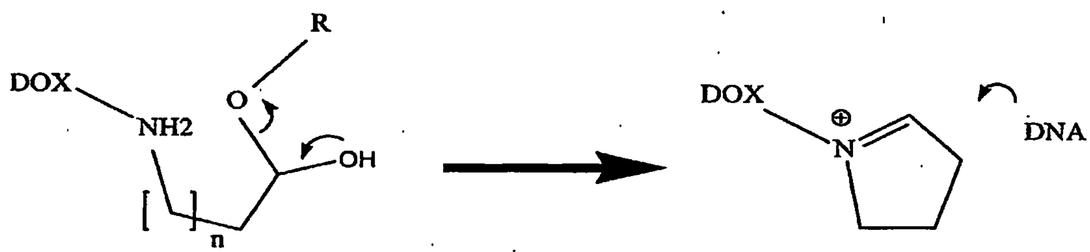
[0063] Part A. *Hypoxia-activated Doxorubicin Derivative Prodrug*. The single free amino group of doxorubicin, daunomycin, epirubicin, and derivatives thereof having only this single free amino group can be readily modified with the hypoxia-activated moieties of the the invention to yield prodrugs of the invention having the following structure (DOX is doxorubicin or one of the related compounds previously mentioned other than the free amino group):



R = alkyl or heteroatom substituted alkyl

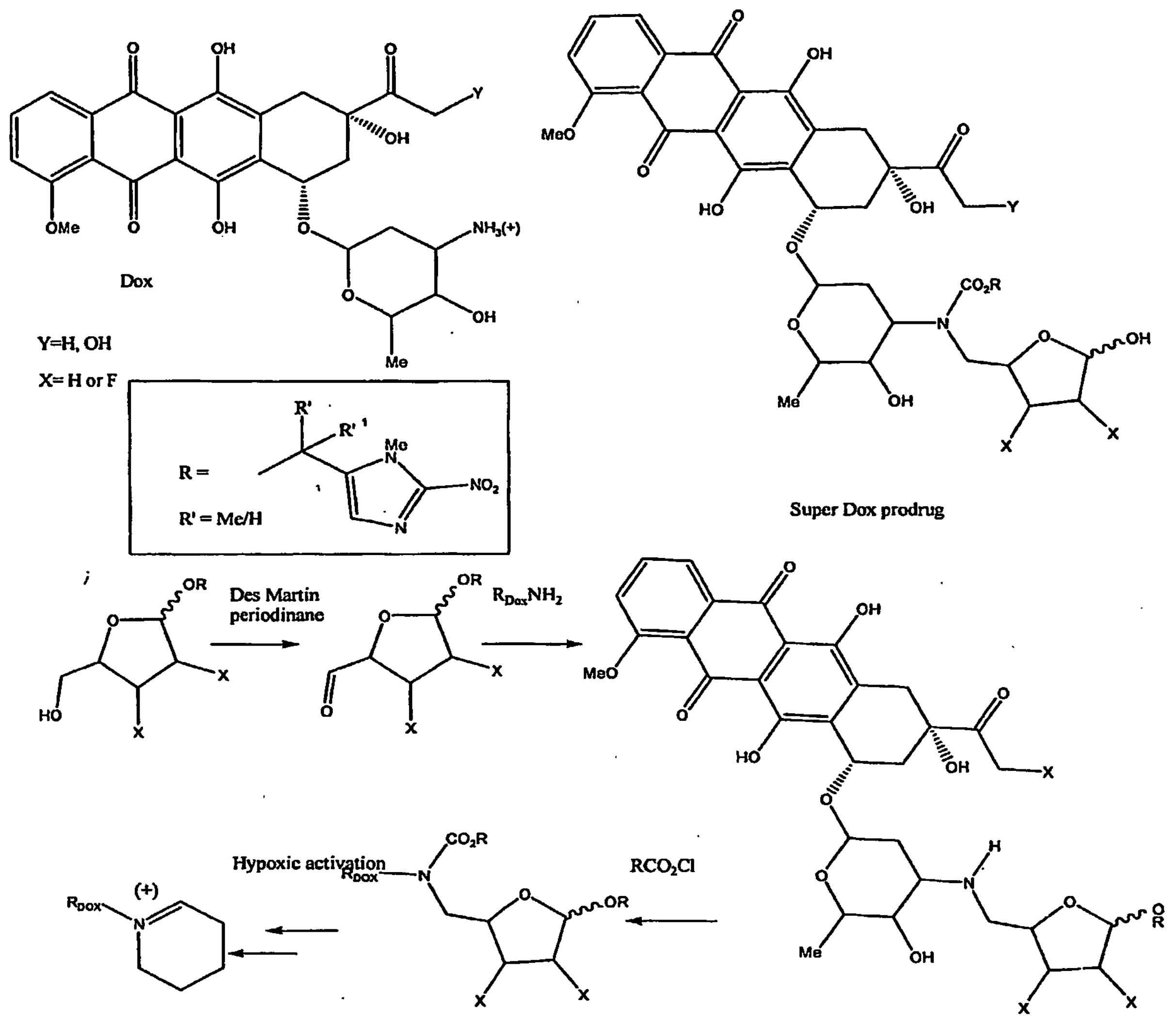
R_1= H, Me

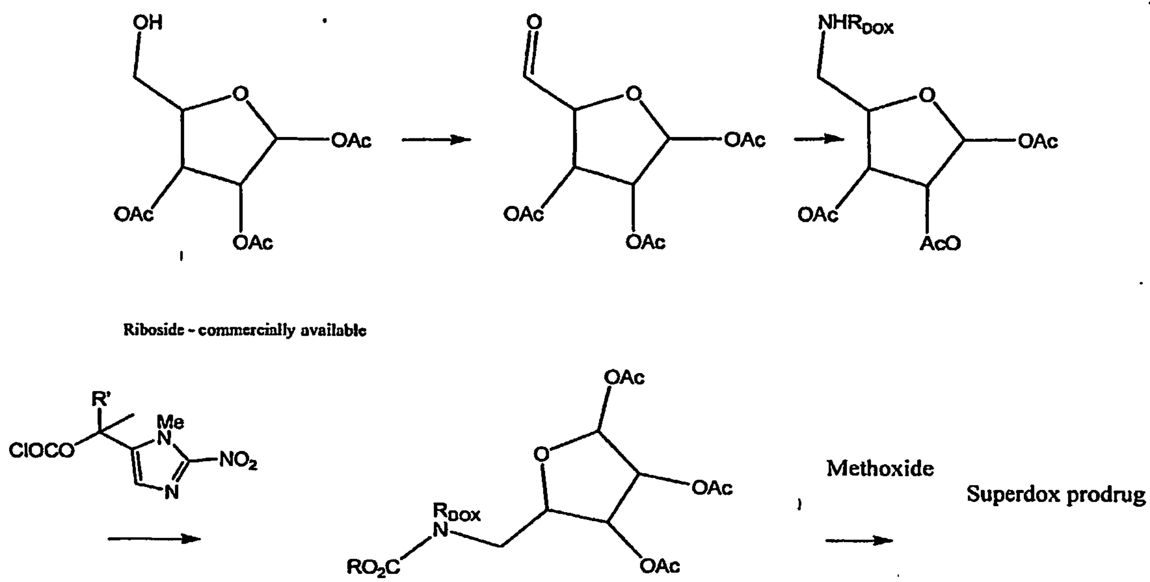
Under hypoxic conditions, the hypoxia-activated moiety is removed, releasing the following highly cytotoxic compound shown as iminium DOX (or Super Dox), below.

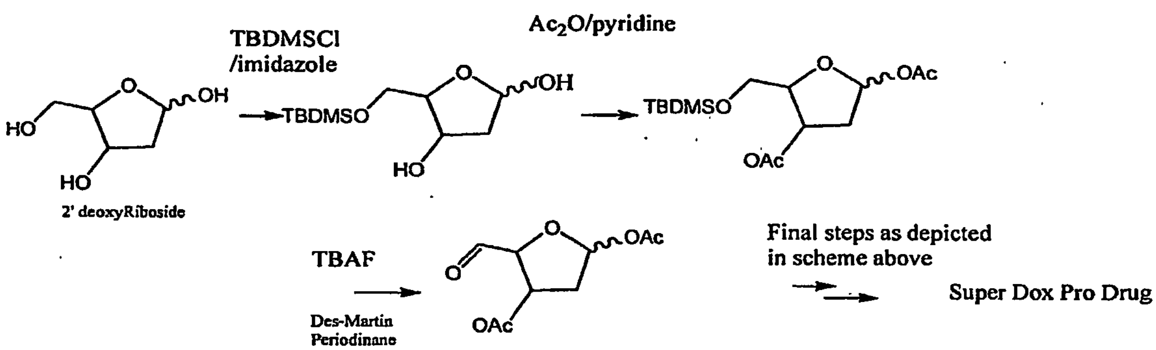


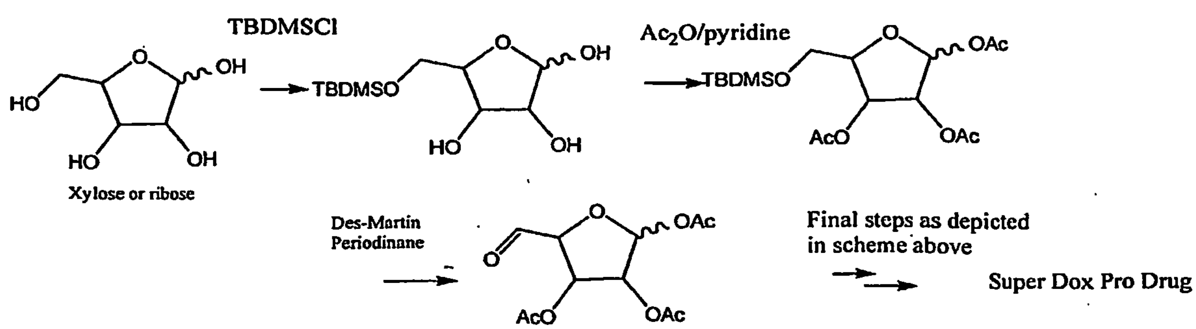
Iminium DOX

The prodrug of the invention can be made by the following scheme.

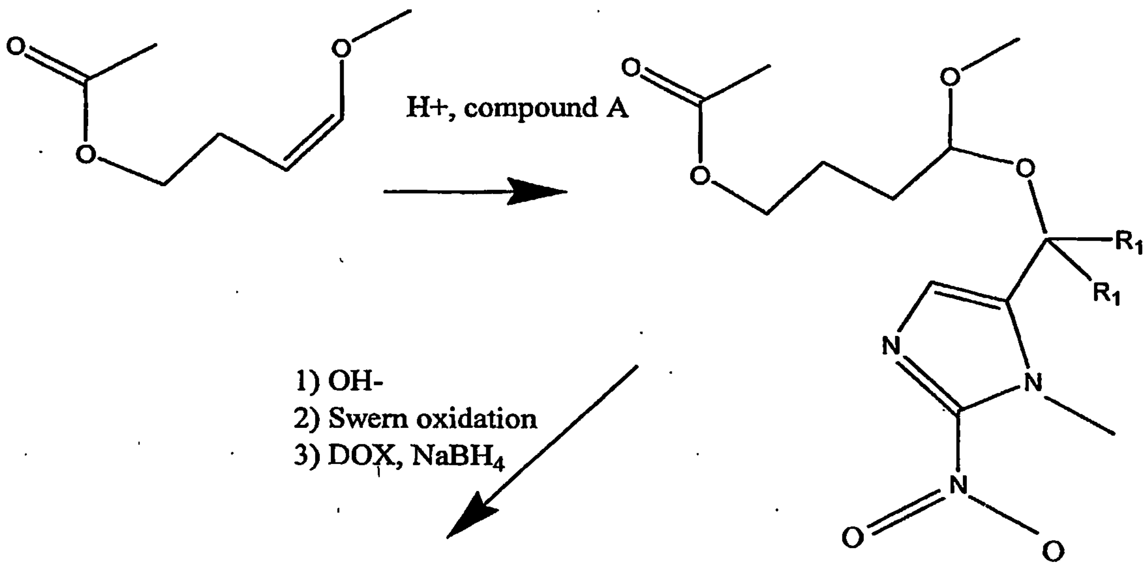

Dox
Y=H, OH
X= H or F
R =
R' = Me/H
NO2
Super Dox prodrug
Des Martin periodinane
RDoxNH2
RCO2Cl
Hypoxic activation





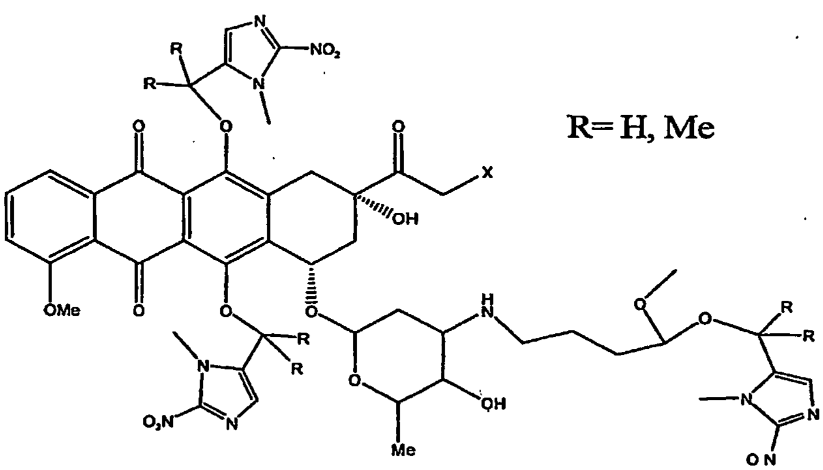


A scheme for another illustrative prodrug of a "super DOX" analog of the present invention is shown below.



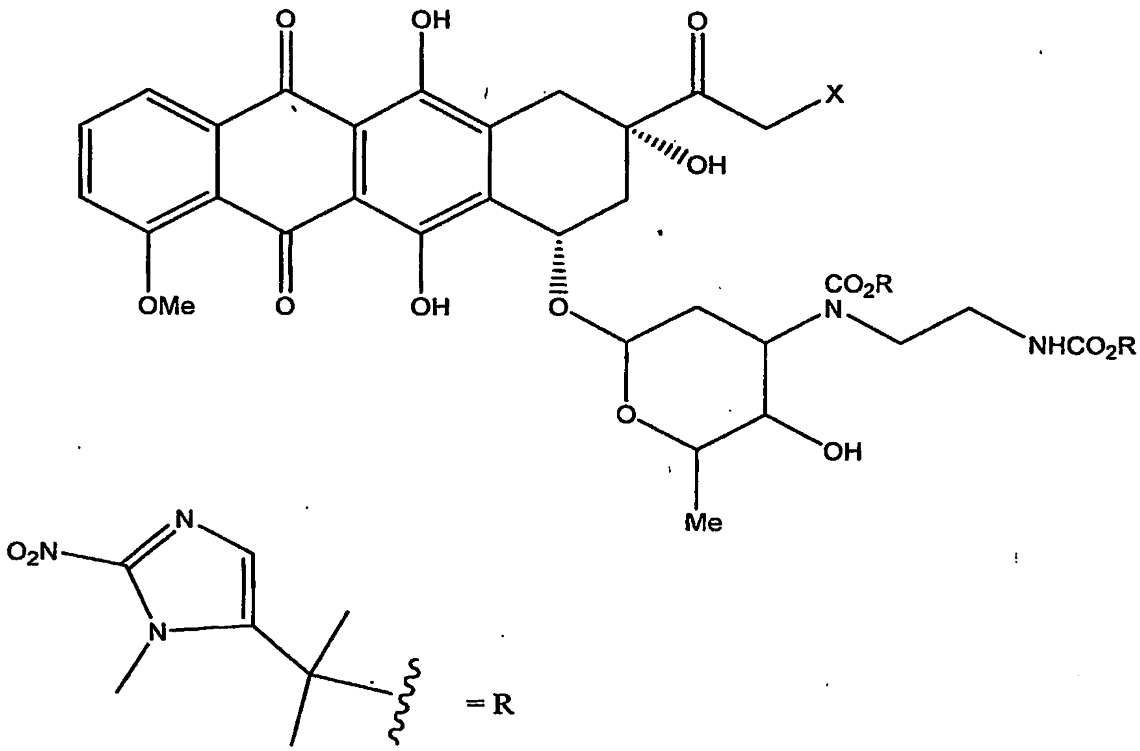
DOX hypoxic prodrug

[0064] Part B. *Hypoxia-activated Doxorubicin Derivative Prodrug that Requires Removal of Multiple Hypoxia-activated Moieties*. The methodology described in part A of this example can be used to generate prodrugs of the invention that have two or more hypoxia-activated moieties. One illustrative prodrug of the invention having three such moieties is shown below (X is defined in part C).



R= H, Me

[0065] Part C. *Hypoxia-activated Doxorubicin Diamine Derivative Prodrug*. Doxorubicin can be derivatized in accordance with a synthetic method of the invention with an aminoethyl moiety to generate a dicationic doxorubicin derivative that is highly cytotoxic and binds more tightly to DNA than doxorubicin. The prodrug, the cytotoxic derivative released by the prodrug, and the method of synthesis of the prodrug are thus embodiments of the present invention. The prodrug of the invention has the following structure (in the structure below, X represents any of the diverse moieties present in doxorubicin, daunomycin, epirubicin, and their naturally occurring and synthetic derivatives):



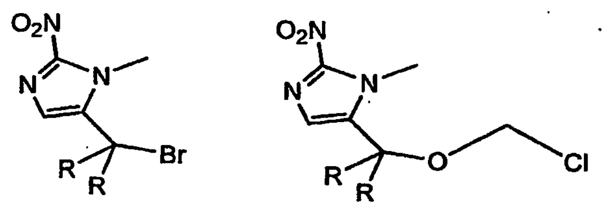
[0066] Upon hypoxia-activated release, the compound is protonated to convert the carboxamates to the corresponding amines. The prodrug is formed by reacting doxorubicin or a doxorubicin derivative with $HC(O)CH_2NHCO_2R$, where R is defined as above, first in the presence of $NaB(OAc)_3H$ and then in the presence of $R'CO_2Cl$, where R' is C_{1-6} lower alkyl, and base.

Example 3

Prodrugs of Etoposide

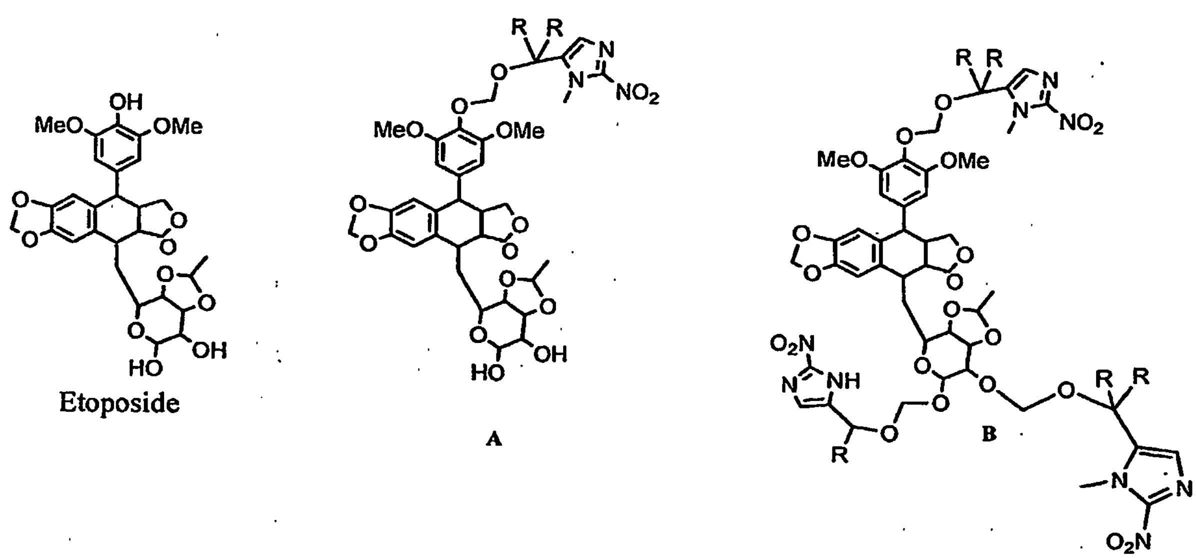
[0067] Etoposide is a potent non-intercalating DNA topoisomerase II inhibitor. In accordance with the synthetic methods of the invention, one can readily form prodrugs of the invention that release etoposide under hypoxic conditions as follows. The hypoxia-activated moiety is attached to the etoposide at the phenolic hydroxy positions via an ether linker.

[0068] These linkers can be provided by other useful intermediate reagents of the invention, shown below. These reagents can be readily prepared from the corresponding alcohol



R=H, Me

[0069] Two illustrative prodrugs, labeled A and B below, and a scheme for forming them from etoposide are shown below.



Etoposide

A

B

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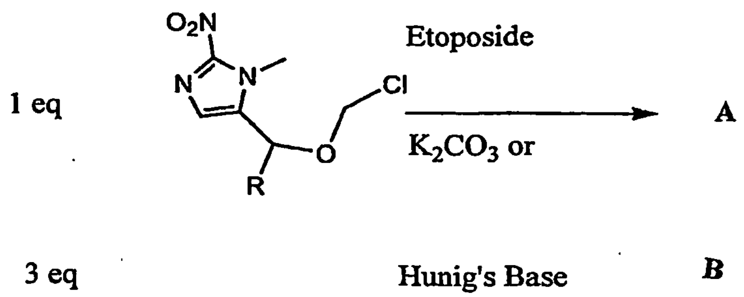
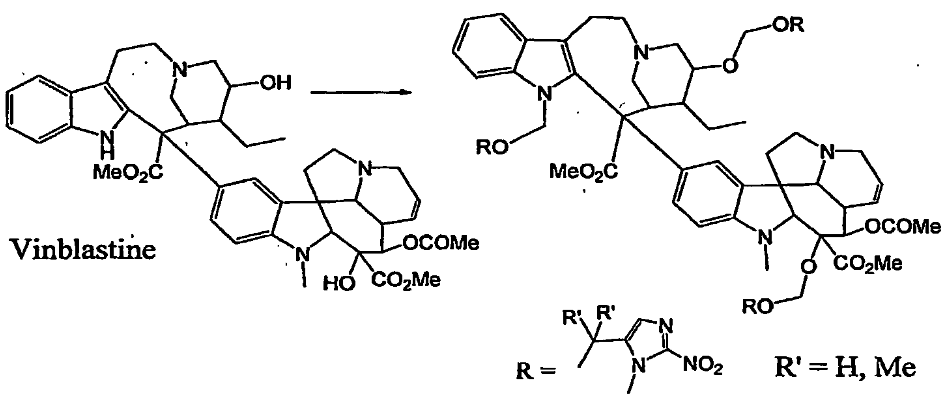


Example 4

Prodrugs of Vinca Alkaloids

[0070] Prodrugs of the invention that release vinblastine (or another vinca alkaloid) can be prepared from vinblastine (or another vinca alkaloid) as shown in the scheme below (the indole NH can be attached to the hypoxia-activated moiety selectively over attachment at the hydroxyls) using synthetic methodology substantially similar to that described in Example 3.



Vincristine-releasing prodrugs of the invention are prepared in an analogous manner, starting with vincristine.

Example 5

Prodrugs of Topotecan

[0071] Prodrugs of the invention that release topotecan can be prepared from topotecan as shown in the scheme below using synthetic methodology substantially similar to that described in Example 3.

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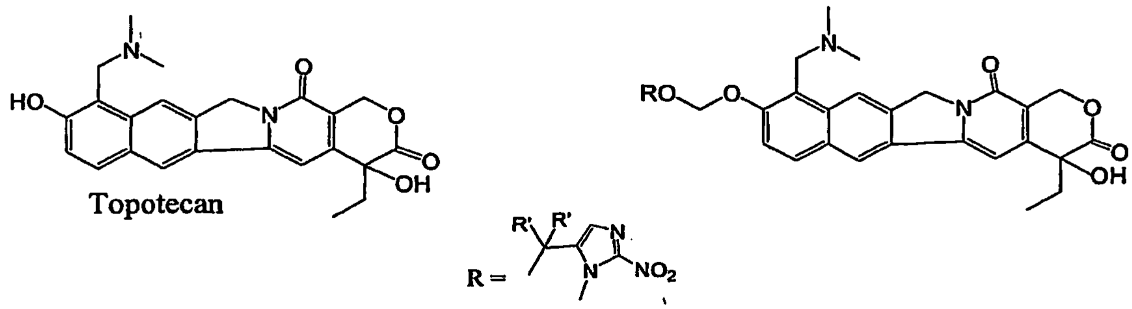
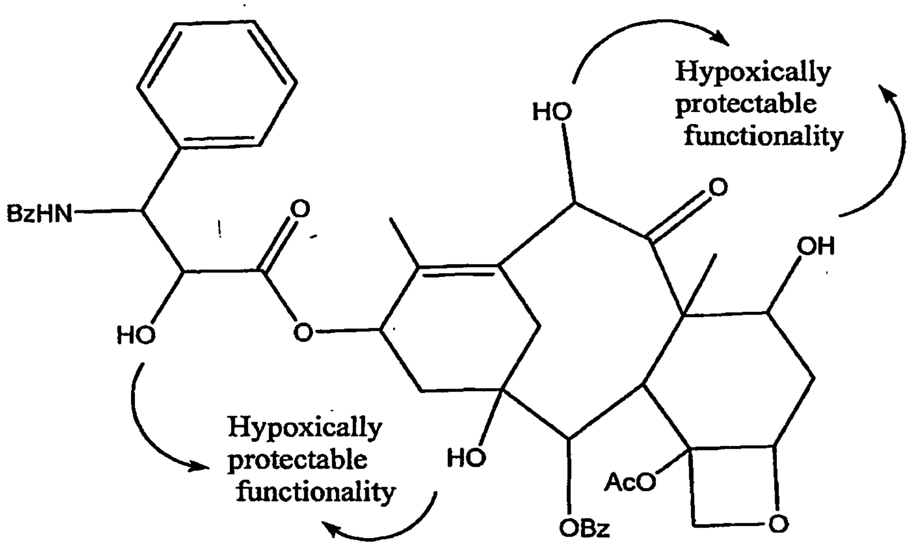


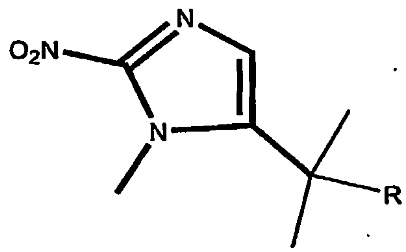
Example 6

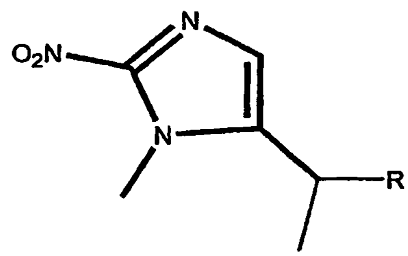
Prodrugs of Taxanes

[0072] Prodrugs of the invention that release paclitaxel, docetaxel, or another taxane can be prepared from paclitaxel (or docetaxel or another taxane) using synthetic methodology substantially similar to that described in Example 3 to prepare compounds similar in structure to the structure shown below.



[0073] As indicated in the structure above, such prodrugs of the invention can have one, two, three, or four hypoxia-activated moieties. In some embodiments, these hypoxia-activated moieties are selected from those shown below.



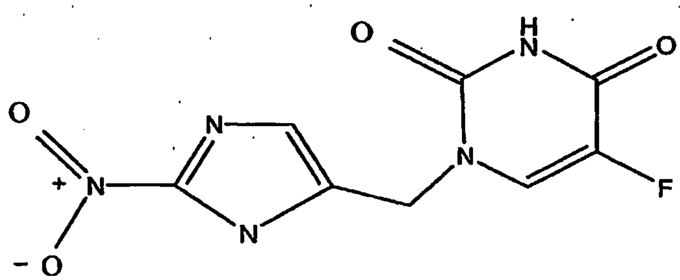


R = OCH_2Cl,

Example 7

Prodrugs of 5-FU

[0074] Prodrugs of the invention that release 5-fluorouracil (5-FU) can be prepared from 5-FU using synthetic methodology substantially similar to that described in Example 3. One such prodrug has the structure shown below.

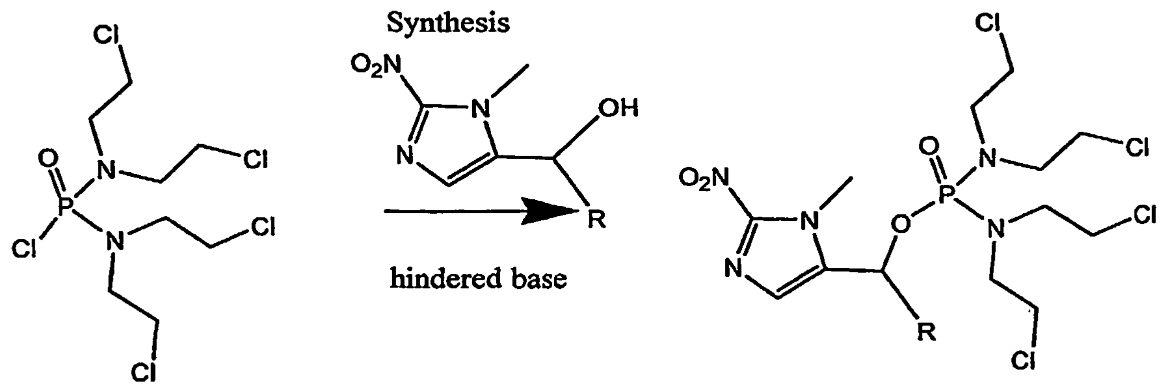


Another prodrug of the invention has two hypoxia-activated moieties attached, one as shown in the structure above, and the other to the other ring nitrogen.

Example 8

Prodrugs of Alkylating Agents

[0075] Prodrugs of the invention that release an alkylating agent can be prepared from as shown in the scheme below using synthetic methodology substantially similar to that described in Example 3. A prodrug of cyclophosphamide provided by the invention is used to illustrate this aspect of the invention. This prodrug of the invention can be synthesized in accordance with the methods of the invention using the following illustrative synthetic method.



Example 9

Prodrugs of Hydroxyurea

[0076] Prodrugs of the invention that release hydroxyurea (HU) can be prepared from hydroxyurea as shown in the scheme below using synthetic methodology substantially similar to that described in Example 3. HU (sold as Hydrea by Bristol-Myers Squibb) is an anti-leukemic drug with a mechanism of action ascribed to the inhibition of the DNA synthesizing enzyme ribonucleoside reductase. HU has also been shown to be effective against brain tumors (meningioma).

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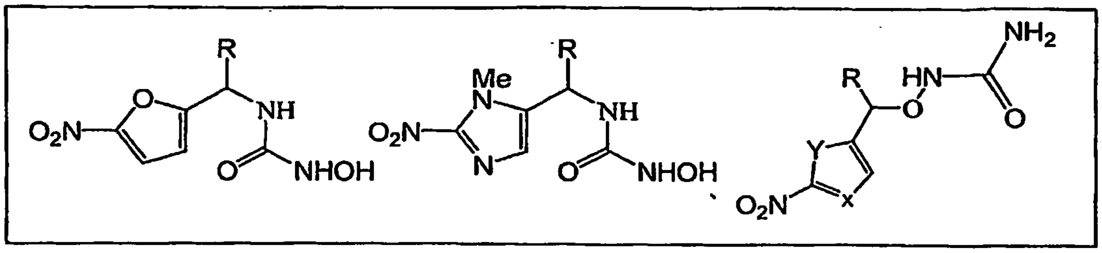
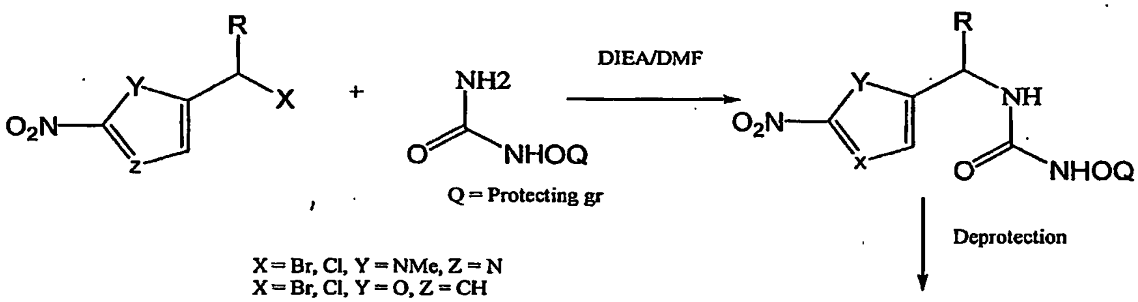
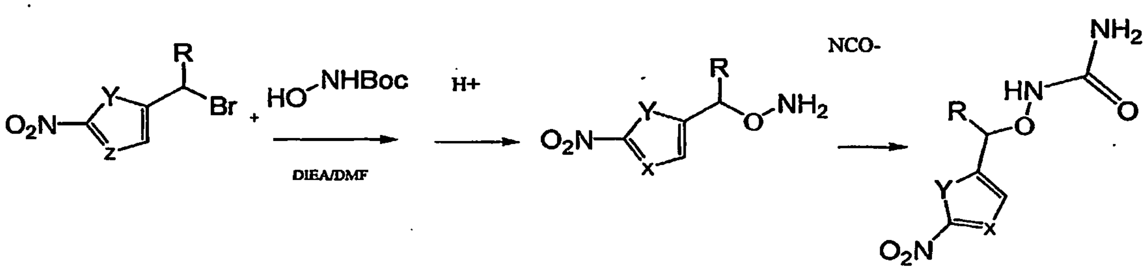


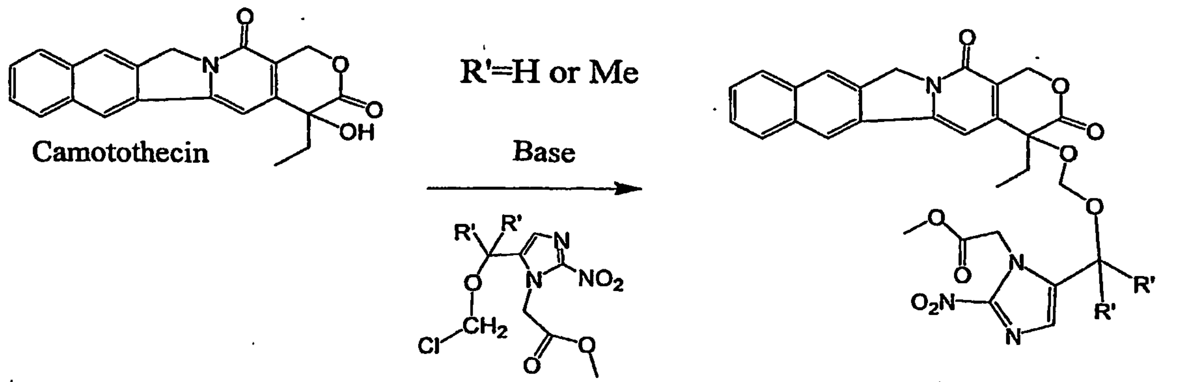




Example 10

Nitroimidazole-20-Camptothecin prodrugs

[0077] A representative example of a method of the invention for preparing an illustrative camptothecin prodrug of the invention is shown below.



Hypoxic triggered prodrug

[0078] The N methylacetate moiety on the imidazole can be synthesized by demethylating the hydroxymethyl imidazole derivative with hydroiodic acid and realkylating it with methyl bromoacetate. Upon prodrug formation, the methoxy ester is removed, optionally with sodium hydroxide followed by acid treatment to restore the active lactone of camptothecin.

[0079] The invention, having been described in summary and detail and illustrated by example, is set forth in the following claims.

What is claimed is:

1. A method for treating cancer in a subject, said method comprising administering to said subject an effective amount of a hypoxia-activated prodrug, wherein said hypoxia-activated prodrug has the formula:

Hyp-L-N

wherein

Hyp is a hypoxic activator;
L is a bond or a linking group; and
N is an anti-neoplastic agent.

2. A method in accordance with claim 1, wherein said hypoxic activator is nitroimidazole.

3. A method in accordance with claim 2, wherein said hypoxic activator comprises of two or more nitroazole moieties.

4. A method in accordance with claim 2, wherein said linking group is a carbamate.

5. A method in accordance with claim 2, wherein said anti-neoplastic agent has an average IC_{50} against the NCI cancer cell line panel of less than 10 nM.

6. The method of Claim 5, wherein said IC_{50} is less than 100 pM.

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ABSTRACT OF THE INVENTION

[0080] Hypoxia-activated prodrugs can be used to treat cancer when administered alone or in combination with one or more anti-neoplastic agent(s).

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